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(54) Title: DERIVATIVES AND ANALOGUES OF 2-DEOXY-2,3-DIDEHYDRO-N-ACETYL NEURAMINIC ACID AND THEIR USE AS ANTIVIRAL AGENTS

(57) Abstract

Derivatives and analogues of 2-deoxy-2,3-didehydro-N-acetyl neuraminic acid, pharmaceutical formulations thereof, methods for their preparation and their use in the treatment of viral infections, in particular influenza, are described.

"DERIVATIVES AND ANALOGUES OF 2-DEOXY-2,3-DIDEHYDRO-N-ACETYL NEURAMINIC ACID AND THEIR USE AS ANTIVIRAL AGENTS".

This invention relates to a new class of chemical compounds and to their use in medicine. In particular the invention concerns new 4-substituted-2-deoxy 2,3-didehydro derivatives of α-D-neuraminic acid, methods for their preparation, pharmaceutical formulations thereof and their use as antiviral agents.

Enzymes with the ability to cleave N-acetyl neuraminic acid (NANA), also known as sialic acid, from other sugars are present in many microorganisms. These include bacteria such as Vibrio cholerae, Clostridium perfringens, Streptococcus pneumoniae, and Arthrobacter sialophilus, and viruses such as influenza virus, parainfluenza virus, mumps virus, Newcastle disease virus, fowl plague virus, and Sendai virus. Most of these viruses are of the orthomyxovirus or paramyxovirus groups, and carry a neuraminidase activity on the surface of the virus particles.

Many of the neuraminidase-possessing organisms are major pathogens of man and/or animals, and some, such as influenza virus, Newcastle disease virus, and fowl plague virus, cause diseases of enormous economic importance.

It has long been thought that inhibitors of neuraminidase activity might prevent infection by neuraminidase-bearing viruses. Most of the known neuraminidase inhibitors are analogues of neuraminic acid, such as 2-deoxy-2,3-didehydro-N-acetylneuraminic acid (DANA) and its derivatives. See, e.g., Meindl et al., Virology 1974 58 457-63. The most active of these is 2-deoxy-2,3-dehydro-N-trifluoroacetyl-neuraminic acid (FANA), which inhibits multi-cycle replication of influenza and parainfluenza viruses in vitro. See Palese et al., Virology 1974 59 490-498.

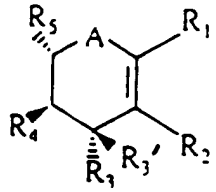
A number of 2-deoxy-2,3-didehydro-N-acetyl-neuraminic acid derivatives are known in the art. See for example P. Meindl et al., Virology, 58, 457-463 (1974); P. Meindl and H. Tuppy, Mh. Chem, 100 (4), 1295-1306 (1969); M. Flashner et al., Carbohydrate Research, 103, 281-285 (1982); E. Zbiral et al., Liebigs Ann Chem, 159-165 (1989); T. Ogawa

produce the β-anomer of 2-deoxy-N-acetylneuraminic acid. This β-anomer did not inhibit Vibrio cholerae neuraminidase.

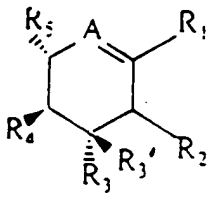
Th most potent in vitro inhibitors of viral neuraminidase have thus been identified as compounds that are based on the neuraminic acid framework, and these are thought by some to be transition-state analogues. Miller et al., Biochem. Biophys. Res. Comm. 1978 83 1479. But while many of the aforementioned neuraminic acid analogues are competitive inhibitors of neuraminidases, to date, none has been reported as showing anti-viral activity in vivo. For example, although a half-planar, unsaturated 6-member ring system has been asserted to be important for inhibitory activity, see Dernick et al. in ANTIVIRAL CHEMOTHERAPY (K. K. Gauri ed.) Academic Press, 1981, at pages 327-336, some compounds characterized by such a system, notably FANA, have been reported not to possess in vivo anti-viral activity. See Palese and Schulman in CHEMOPROPHYLAXIS AND VIRUS INFECTION OF THE UPPER RESPIRATORY TRACT, Vol. 1 (J. S. Oxford ed.) CRC Press, 1977, at pages 189-205.

We have now found novel 4-substituted 2-deoxy-2,3-didehydro derivatives of α-D-neuraminic acid which are active in vivo.

The invention therefore provides in a first aspect compounds of formula (I) or formula (Ia)



(I)



(Ia)

where in general formula (I), A is oxygen, carbon or sulphur, and in general formula (Ia), A is nitrogen or carbon;

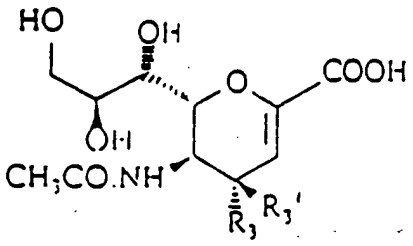
(i) when $R^3$ or $R^{3'}$ is $OR^6$ or hydrogen, and A is nitrogen, then said compound cannot have both

(a) an $R^2$ that is hydrogen, and

(b) an $R^4$ that is NH-acyl, and

(ii) $R^6$ represents a covalent bond when Y is hydrogen.

In a preferred embodiment, the compound has general formula (II)




(II)

i.e. in general formula (I) above, $R^1$ is COOH, $R^2$ is hydrogen, $R^4$ is acetamido, and $R^5$ is $-CHOH.CHOH.CH_2OH$, and $R^3$ is hydrogen or $R^{3'}$, where $R^{3'}$ denotes $-N_3$, $-CN$, $-CH_2NH_2$, or $-N.R^8.R^9$;

$R^8$ and $R^9$ are the same or different, and each denotes hydrogen, a linear or cyclic alkyl group of 1 to 6 carbon atoms, an acyl or substitued acyl group of 1 to 6 carbon atoms, $-C.(NH).NH_2$, $-CH_2.COOH$, $-CH_2CH_2-OH$ or $-CH_2.CH.(R^{10})(R^{11})$,

$R^{10}$ and $R^{11}$ may be the same or different, and each denotes oxygen or $R^{12}N=$, and

$R^{12}$ denotes hydrogen, $-OH$, $-OCH_3$, $-NH_2$, or $(CH_3)_2N-$.

We have found a particular subclass of compounds of formula (I) which are unexpectedly more active than their corresponding 4-hydroxy analogues.

Thus in a particularly preferred aspect the invention provides compounds of formula (Ib)

isopropyl, t-butyl) alkyl groups.

By pharmaceutically acceptable derivative is meant any pharmaceutically acceptable ester or salt of such ester of the compounds of formula (I) or any other compound which upon administration to the recipient is capable of providing (directly or indirectly) a compound of formula (I) or an antivirally active metabolite or residue thereof.

It will be appreciated by those skilled in the art that the compounds of formula (I) may be modified to provid pharmaceutically acceptable derivatives thereof at any of the functional groups in the compounds. Of particular interest as such derivatives are compounds modified at the C-1 carboxyl function, the C-7 or C-9 hydroxyl functions, or at amino groups. Thus compounds of interest include $C_{1-4}$alkyl (such as methyl, ethyl or propyl e.g. isopropyl) or aryl (e.g. phenyl, benzoyl) esters of the compounds of formula (I), C-7 or C-9 esters of compounds of formula (I) such as acetyl esters thereof, C-7 or C-9 ethers such as phenyl ethers, benzyl ethers, p-tolyl ethers, and acylated amino derivatives such as formyl, acetamido.

It will be appreciated by those skilled in the art that the pharmaceutically acceptable derivatives of the compounds of formula (I) may be derivatised at more than one position.

Pharmaceutically acceptable salts of the compounds of formula (I) include those derived from pharmaceutically acceptable, inorganic and organic acids and bases. Examples of suitable acids include hydrochloric, hydrobromic, sulphuric, nitric, perchloric, fumaric, maleic, phosphoric, glycollic, lactic, salicylic, succinic, toluene-p-sulphonic, tartaric, acetic, citric, methanesulphonic, formic, benzoic, malonic, naphthalene-2-sulphonic and benzenesulphonic acids. Other acids such as oxalic, while not in themselves pharmaceutically acceptable, may be useful in the preparation of salts useful as intermediates in obtaining compounds of the invention and their pharmaceutically acceptable acid addition salts.

Salts derived from appropriate bases include alkali

that ref rence herein to treatment extends to prophylaxis as well as the treatment of established infections or symptoms.

It will be further appreciated that the amount of a compound of the invention required for use in treatment will vary not only with the particular compound selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient, and will ultimately be at the discretion of the attendant physician or veterinarian. In general however, a suitable dose will be in the range of from about 0.01 to 750mg/kg of bodyweight per day preferably in the range of 0.1 to 100 mg/kg/day, most preferably in the range of 0.5 to 25 mg/kg/day.

In particular we have found that the effective doses of the compounds tested are related to their in vitro potency. Thus DANA (which has $IC_{50}$ plaque reduction of 5µg/ml) has been found to be effective at doses of between 1 and 10mg/kg per treatment. The corresponding methyl ester of DANA ($IC_{50}$ 50-100µg/ml) is effective at proportionally higher dose.

Treatment is preferably commenced before or at the time of infection and continued until virus is no longer present in the respiratory tract. However the compounds are also effective when given post-infection, for example after the appearance of established symptoms.

Suitably treatment is given 1-4 times daily and continued for 3-7, e.g. 5 days post infection depending upon the particular compound used.

The desired dose may be presented in a single dose or as divided doses administered at appropriate intervals, for example as two, three, four or more sub-doses per day.

The compound is conveniently administered in unit dosage from for example containing 10 to 1500mg, conveniently 20 to 1000mg, most conveniently 50 to 700mg of active ingredient per unit dosage form.

While it is possible that, for use in therapy, a compound of the invention may be administer d as the raw chemical, it is preferable to present the active ingredient

dropper or pipette this may be achieved by the patient administering an appropriate, predetermined volume of the solution or suspension. In the case of a spray this may be achieved for example by means of a metering atomising spray pump.

Intranasal administration may also be achieved by means of an aerosol formulation in which the compound is provided in a pressurised pack with a suitable propellant such as a chlorofluorocarbon (CFC), for example dichlorodifluoromethane, trichlorofluoromethane or dichlorotetrafluroroethane, carbon dioxide or other suitable gas. The aerosol may conveniently also contain a surfactant such as lecithin. The dose of drug may be controlled by provision of a metered valve.

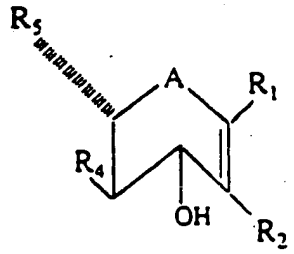
Alternatively the compounds may be provided in the form of a dry powder, for example a powder mix of the compound in a suitable powder base such as lactose, starch, starch derivatives such as hydroxypropylmethyl cellulose and polyvinylpyrrolidine (PVP). Conveniently the powder carrier will form a gel in the nasal cavity. The powder composition may be presented in unit dose form, for example in capsules or cartridges of e.g. gelatin or blister packs from which the powder may be administered by means of an inhaler.

In the intranasal formulations the compound will generally have a small particle size, for example of the order of 5 microns or less. Such a particle size may be obtained by means known in the art, for example by micronisation.

When desired the formulations may be adapted to give sustained release of the active ingredient. The compounds of the invention may also be used in combination with other therapeutic agents, for example other anti-infective agents. In particular the compounds of the invention may be employed with other antiviral agents. The invention thus provides in a further asp ct a combination comprising a compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof tog ther with another

wher in $R^2$ is as defined in formula (I), and L is a leaving group (for example a sulphonic acid residue such as tosyl, mesyl, trifluoromesyl) or a protected derivative thereof is reacted with the appropriate nucleophile, for example azide, cyanide, an appropriate carbanion, or thioacetate.

The compounds of formula (III) may be obtained from the corresponding compounds of formula (IV)




(IV)

by inversion of the 4-OH group by methods known in the art, for example by reaction with a Lewis acid (such as $BF_3$ etherate) followed by hydrolysis. The compounds of formula (IV) are either known in the art or may be obtained by methods analogous to those for preparing the known compounds.

In a second method (B) the compounds of formula (I) may be prepared from other compounds of formula (I) by interconversion. Thus compounds of formula (I) wherein $R^3$ is $NH_2$ or $CH_2NH_2$ may be prepared by reduction of the corresponding azido or cyano analogues respectively.

Compounds wherein $R^3$ is NH alkyl or guanidino may be prepared by derivatisation of the corresponding compound wherein $R^3$ is $NH_2$.

Compounds of formula I where $R^1$ is COOH may be prepared by hydrolysis of the corresponding ester under either acidic or basic conditions, for example at pH 11-12 (using a base such as sodium or ammonium hydroxide), or at pH 2-3 (using an acid such as sulphuric acid).

As will be appreciated by those skilled in the art, it may be necessary or desirable at any stage in the above described processes to protect one or more sensitive groups in the molecule to prevent undesirable side reactions; the protecting group may be removed at any conv nient subsequent stage in the reaction sequence.

General Methodologies

The following general methods are appliicable to the synthesis of compounds of the invention.

Deacetylation

Treatment of the acetylated material with Amberlite IRA-400 ($OH^-$) with stirring, for a period of time, generally 2-3 h, at room temperature results in complete de-O-acetylation. The resin is filtered off and the filtrate concentrated to dryness to afford the desired de-O-acetylation material.

Those skilled in the art would recognise that other standard procedures are available for the complete de-O-acetylation of the same material, such as treatment with sodium methoxide in methanol.

Deesterification

The completely de-O-acetylated material is taken up in aqueous sodium hydroxide and stirred at room temperature for a period of time, generally 2-3 h. The mixture is then adjusted to pH 7.0-7.5 with Dowex 50w X 8 ($H^+$) resin. Filtration followed by freeze-drying of the filtrate affords the desired deesterified material.

Those skilled in the art would readily be able to identify several alternative options for the deesterification of the same material such as acid hydrolysis, alternative base hydrolyses e.g. ammonium hydroxide, potassium hydroxide.

Intermediate compounds referred to in Examples 1 to 15 are identified as follows:

COMPOUND 2

Methyl 5-acetamido-7,8,9-tri-O-acetyl-2,3,5-trideoxy-D-glycero-D-talo-non-2-enopyranosonate (4-epi-Neu5,7,8,9$Ac_4$2en1Me)

COMPOUND 19

Methyl 5-acetamido-4-N-methylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N-methylamino-Neu5Ac2en1Me)

COMPOUND 21

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-N,N-dimethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2enopyranosonate (4-N,N-dimethylamino-Neu5,7,8,9$Ac_4$2en1Me)

COMPOUND 22

Methyl 5-acetamido-4-N,N-dimethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N,N-dimethylaminoNeu5Ac2en1Me)

COMPOUND 24

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-N-methoxycarbonylmethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N-methoxycarbonylmethylaminoNeu5,7,8,9$Ac_4$2en1Me)

COMPOUND 25

Methyl 5-acetamido-4-N-methoxycarbonylmethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N-methoxycarbonylmethylaminoNeu5Ac2en1Me)

COMPOUND 27

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-N-2′-hydroxyethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N-2′-hydroxyethylaminoNeu5,7,8,9-$Ac_4$2en1Me)

COMPOUND 28

Methyl 5-acetamido-4-N-2′-hydroxyethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N-2′-hydroxyethylaminoNeu5,7,8,9$Ac_4$2en1Me)

COMPOUND 29

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-N-2′-hydroxyethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N-2′-hydroxyethylaminoNeu5Ac2en1Me)

COMPOUND 30

3-Deoxy-D-glycero-D-galacto-2-nonulopyranosonic acid (KDN)

Preparation of (2)

To an agitated solution of m thyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2,3,5-trideoxy-D-glycero-D-galacto-non-2-enopyranosonate (1) (1500 mg, 3.17 mmol) in a mixture of benzene (50 ml) and methanol (300 mg) was added dropwise $BF_3Et_2O$ (12 ml) over thirty minutes under a nitrogen atmosphere at room temperature. The whole mixture was then allowed to stir at room temperature for 16 hours. The solution was diluted with ethyl acetate (250 ml), washed successively with saturated $NaHCO_3$ solution (30 ml x 3) and water (20 ml x 3), then evaporated to a small volume (about 10 ml), to which was added water (0.5 ml) and acetic acid (0.5 ml). The whole mixture was then stirred at room temperature for two days before being diluted with ethyl acetate (200 ml). The ethyl acetate solution was washed with 5% $NaHCO_3$ solution (30 ml x 2) and water (20 ml x 3), then evaporated to dryness. The residue was chromatographed (silica gel, ethyl acetate as eluting solvent) to afford pure compound (2) (550 mg, 40%).

$^1$H-nmr ($CDCl_3$) $\delta$ (ppm); 1.95, 2.06, 2.08, 2.10, 2.35 (s, 15H, Acetyl $CH_3$ x 5), 3.80 (s, 3H, $COOCH_3$), 4.1-4.4 (m, 4H, $H_4$, $H_5$, $H_6$, $H_9$), 4.82 (dd, 1H, $J_{9,8}$ 1.8Hz, $J_{9,9}$, 12.3Hz, $H_9$), 5.27 (m, 1H, $H_8$), 5.45 (dd, 1H, $J_{7,8}$ 3.5Hz, $H_7$), 6.15 (d, 1H, $J_{3,4}$ 5.4Hz, $H_3$), 6.47 (d, 1H, $J_{NH,5}$ 8.8Hz, -CONH).

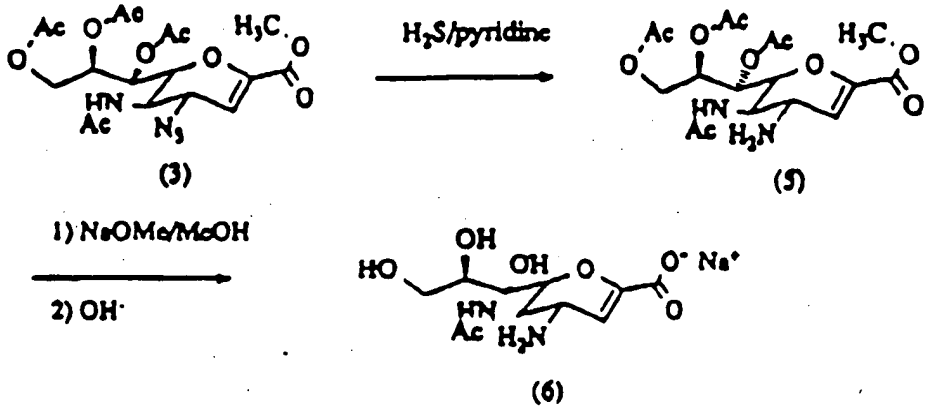
Preparation of (3)

To a stirred solution of compound (2) (800 mg, 1.67 mmol) in anhydrous dichloromethane (10 ml) and dry pyridine (316 mg, 4 mmol) at -30° to -40°C, was added dropwise a solution of trifluoromethane sulphonic anhydride ($Tf_2O$) (556 mg, 2 mmol) in dichloromethane (2 ml) over 15 minutes. The reaction mixture was then stirred at -30° for 5 hours, and concentrated to dryness in vacuo. The residue was then dissolved in dry DMF (5 ml) containing a mixture of sodium azide (650 mg, 10 mmol) and tetrabutylammonium hydrogen sulphate (170 mg, 0.5 mmol). The reaction mixture was stirred at room temperature for 16 hours, and then evaporated

i.r. (KBr)$cm^{-1}$ 3400 (br.-OH), 2100 ($-N_3$), 1714 (carbonyl). $^1$H-nmr ($D_2O$) δ (ppm). 2.06 (5, 3H, acetyl $CH_3$), 3.64 (dd, 1H, $J_{9',8}$ 6.3Hz, $J_{9',9}$ 11.8Hz, $H_{9'}$), 3.65 (dd, 1H, $J_{7,6}$ 3.9Hz, $J_{7,8}$ 6.8Hz, $H_7$), 3.88 (dd, 1H, $J_{9,8}$ 2.6Hz, $J_{9,9'}$ 11.8Hz, $H_9$), 3.94 (m, 1H, $J_{8,7}$ 6.8Hz, $J_{8,9}$ 2.6Hz, $J_{8,9'}$ 6.3Hz, $H_8$), 4.21 (dd, 1H, $J_{5,4}$ 10.4Hz, $J_{5,6}$ 8.9Hz, $H_5$), 4.31 (dd, 1H, $J_{4,3}$ 2.2Hz, $J_{4,5}$ 2.2Hz, $J_{4,5}$ 10.4Hz, $H_4$), 4.34 (dd, 1H, $J_{6,5}$ 8.9Hz, $J_{6,7}$ 3.9Hz, $H_6$) 5.82 (d, 1H, $J_{3,4}$ 2.2Hz, $H_3$).

Example 2 The preparation of Sodium 5-Acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-amino-Neu5Ac2en) (6)

The overall reaction scheme is as follows:

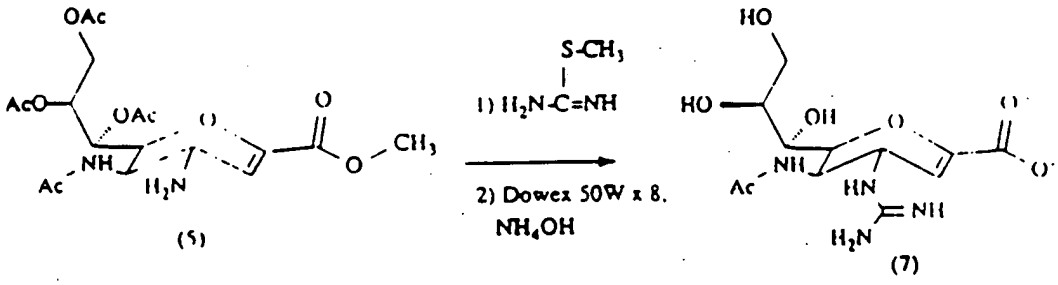



Preparation of (5)

Into a solution of methyl 5-acetamido-7,8,9-tri-O-acetyl-4-azido-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (3) prepared as in Example 1, (95 mg, 0.208 mmol) in pyridine (6 ml) was bubbled with $H_2S$ for 16 hours at room temperature. The solution was then flushed with nitrogen for 15 minutes, and evaporated to remove pyridine under high vacuum. The residue was chromatographed (silica gel, ethyl acetate/isopropanol/water = 5/2/1) to afford a colourless compound (5) (50 mg, 56%).

Example 3 The preparation of Ammonium 5-Acetamido-4-guanidino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (7)

The overall reaction scheme is as follows:




Into a solution of S-methylisourea (546 mg, 3 mmol) in water (15 mL) at ice-bath temperature, methyl-5,7,8,9-tri-O-acetyl-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (5) prepared as in Example 2 (40 mg, 0.093 mmol) was added. The reaction mixture was stirred at 5°C for seven days and poured onto a column of Dowex 50W X 8 ($H^+$) resin (35 mL). The column was then washed with cold water (700 mL) and eluted with 1.5 M $NH_4OH$ solution. The eluate (120 mL) was concentrated to dryness under high vacuum. The resulting residue was chromatographed (silica gel; solvent system 1: ethyl acetate/isopropanol/water, 1/5/1; solvent system 2: 75% isopropanol) to provide the title compound (7) (8 mg, 24.5%).

Compound (7) gave a strong, positive Sakaguchi reaction, indicating the presence of a guanidine group. NMR data for compound (7) are given below. $^1$H-nmr ($D_2O$ + $CD_3OD$) δ (ppm).

2.06 (s, 2H, acetyl $CH_3$), 3.60 (br. d., 1H, $J_{7,8}$ 9.4Hz, $H_7$), 3.63 (dd, 1H, $J_{9',8}$ 6.2Hz, $J_{9',9}$ 11.8Hz, $H_{9'}$), 3.76 (br. d., 1H, $J_{4,5}$ 9.4Hz, $H_4$), 3.87 (dd, 1H, $J_{9,8}$ 2.6Hz, $J_{9,9'}$, 11.8Hz, $H_9$), 3.93 (ddd, 1H, $J_{8,7}$ 9.4Hz, $J_{8,9}$ 2.6Hz, $J_{8,9'}$ 6.2Hz, $H_8$), 4.01 (dd, 1H, $J_{5,4}$ 9.4Hz, $J_{5,6}$ 10.6Hz, $H_5$), 4.20 (br. d., 1H $J_{6,5}$ 10.6Hz, $H_6$), 5.63 (d, 1H, $J_{3,4}$ 2.1Hz, $H_3$).

and methyl 5-acetamido-7,8,9-tri-O-acetyl-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2- nopyranosonate (5) (90mg, 0.209mmol) in acetonitrile (5mL), was added silver carbonate (116mg, 0.418mmol). The mixture was stirred and protected from light at room temperature for 16 h. The resulting suspension was filtered, and the filtrate was evaporated to dryness. The residue was subjected to flash-column chromotography silica gel, ethyl acetate containing 10% methanol) to afford methyl 5-acetamido-7,8,9-tri-O-acetyl-4-N,N-diallylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (8) (85mg, 80%).

$^1$H-nmr ($CDCl_3$) δ (ppm) 1.94, 2.05, 2.06, 2.11 (s, 12H, acetyl $CH_3$ x 4), 2.97 (dd, 2H, $J_{10a,10b}$ & $J_{10'a,10'b}$ 14.3Hz, $J_{10a,11}$ & $J_{10'a,11'}$ 7.6Hz, $H_{10a}$ & $H_{10'a}$), 3.24 (dd, 2H, $J_{10b,10a}$ & $J_{10'b,10'a}$ 14.3Hz, $J_{10b,11}$ & $J_{10'b,11'}$ 4.9Hz, $H_{10b}$ & $H_{10'b}$), 3.58 (dd, 1H, $J_{4,3}$ 2.4Hz, $J_{4,5}$ 9.3Hz, $H_4$), 3.79 (s, 3H, $COOCH_3$), 4.12-4.26 (m, 3H, $H_6$, $H_{9'}$, $H_5$), 4.70 (dd, 1H, $J_{9,8}$ 2.6Hz, $J_{9,9'}$ 12.3Hz, $H_9$), 5.09 (dd, 2H, $J_{12cis,11}$ & $J_{12'cis,11'}$ 10.6Hz, $J_{12gem}$ & $J_{12'gem}$ ~1.5Hz, $H_{12cis}$ & $H_{12'cis}$), 5.14 (dd, 2H, $J_{12trans,11}$ & $J_{12'trans,11'}$ 17.7Hz, $J_{12gem}$ & $J_{12'gem}$ ~1.5Hz, $H_{12trans}$ & $H_{12'trans}$), 5.27-5.32 (m, 2H, $H_8$ & -CONH-), 5.55 (dd, 1H, $J_{7,6}$ 2.1Hz, $J_{7,8}$ 4.7Hz, $H_7$), 5.72 (m, 2H, $H_{11}$ & $H_{11'}$), 6.07 (d, 1H, $J_{3,4}$ 2.4Hz, $H_3$).

Compound (8) (80mg, 0.156mmol) was dissolved in anhydrous methanol (10mL) containing sodium methoxide (16.2mg, 0.30mmol).

The solution was stirred at room temperature for 2 h, then evaporated to dryness. The residue was taken up in water (5mL), and left at room temperature for 2 h. The resulting solution was neutralized with Dowex 50 x 8 ($H^+$) and freeze-dried to afford the title compound (9) (49mg, 80%).

$^1$H-nmr (D2O) δ (ppm) 1.94 (s, 3H, Acetyl $CH_3$), 3.24-3.44 (m, 4H, $H_{10}$ x 2 & $H_{10'}$ x 2), 3.48-4.33 (m, 7H, $H_4$, $H_5$, $H_6$, $H_7$, $H_8$, $H_9$ & $H_{9'}$), 5.24-5.29 (m, 4H, $H_{12}$ x 2 & $H_{12'}$ x 2), 5.69 (d, 1H, $J_{3,4}$ ~2Hz, $H_3$), 5.73-5.76 (m, 2H, $H_{11}$ & $H_{11'}$)

$^1$H-nmr ($CDCl_3$) of compound (10) is shown as follows δ (ppm) 1.96, 2.05, 2.06, 2.11(s, 12H, Acetyl $CH_3$ x 4), 3.25 (dd, 1H, $J_{10a,10b}$-14.1Hz, $J_{10a,11}$ 5.8Hz, $H_{10a}$), 3.37 (dd, 1H, $J_{10b,10a}$-14.1Hz, $J_{10b,11}$ 5.9Hz, $H_{10b}$), 3.43 (dd, 1H, $J_{4,3}$ 3.1Hz, $J_{4,5}$ 7.5Hz, $H_4$), 3.79 (s, 3H, $COOCH_3$), 4.09 (ddd, 1H, $J_{5,4}$ 7.5Hz, $J_{5,NH9}$·1Hz, $J_{5,6}$ 8.1Hz, $H_5$), 4.21 (dd, 1H, $J_{9',8}$ 7.1Hz, $J_{9',9}$-12.2Hz, $H_{9'}$), 4.30 (dd, 1H, $J_{6,5}$ 8.1Hz, $J_{6,7}$ 4.1Hz, $H_6$), 4.63 (dd, 1H, $J_{9,8}$ 3.2Hz, $J_{9,9'}$-12.2Hz, $H_9$), 5.09 (dd, 1H, $J_{12cis,11}$ 10.2Hz, $J_{12cis,12trans}$-1.3Hz, $H_{12cis}$), 5.18 (dd, 1H, $J_{12trans,11}$ 17.1Hz, $J_{12trans,12cis}$-1.3Hz, $H_{12trans}$), 5.36 (ddd, 1H, $J_{8,7}$ 4.2Hz, $J_{8,9}$ 3.2Hz, $J_{8,9'}$ 7.1Hz, $H_8$), 5.57 (dd, 1H, $J_{7,6}$ 4.1Hz, $J_{7,8}$ 4.2Hz, $H_7$), 5.65 (d, 1H, $J_{NH,5}$ 9.1Hz, -CONH-), 5.83 (dddd, 1H, $J_{11,12trans}$ 17.1Hz, $J_{11,12cis}$ 10.2Hz, $J_{11,10a}$ 5.8Hz, $J_{11,10b}$ 5.9Hz, $H_{11}$), 6.09 (d, 1H, $J_{3,4}$ 3.1Hz, $H_3$).

Compound (10) (50mg, 0.11mmol) was stirred in anhydrous methanol (5mL) containing sodium methoxide (12mg, 0.225mmol) at room temperature for 2 h, then evaporated to dryness. The residue was redissolved in water (5mL) and allowed to stand at room temperature for 2 h before being neutralized with Dowex 50 x 8 ($H^+$) resin. The aqueous solution was freeze-dried to afford compound (11) (31mg, 78%).

$^1$H-nmr (D2O) δ (ppm) 2.02 (s, 3H, $CH_3CO$), 3.42 (dd, 1H, $J_{10a,10b}$-13.4Hz, $J_{10a,11}$ 6.6Hz, $H_{10a}$), 3.52 (dd, 1H, $J_{10b,10a}$-13.4Hz, $J_{10b,11}$ 6.3Hz, $J_{10b}$), 3.51-4.27 (m, 7H, $H_4$, $H_5$, $H_6$, $H_7$, $H_8$, $H_9$ & $H_{9'}$), 5.30 (dd, 1H, $J_{12cis,12trans}$ ~1.5Hz, $J_{12cis,11}$ 10.3Hz, $H_{12cis}$), 5.34 (dd, 1H, $J_{12trans,12cis}$~1.5Hz, $J_{12trans,11}$ 17.7Hz, $H_{12trans}$), 5.72 (d, 1H, $J_{3,4}$ 2.4Hz, $H_3$), 5.89 (dddd, $J_{11,10a}$ 6.6Hz, $J_{11,10b}$ 6.3Hz, $J_{11,12cis}$ 10.3Hz, $J_{11,12trans}$ 17.7Hz, $H_{11}$).

ethyl acetate (50mL) and water (15mL), with the organic layer washed successively with water (5mL x 2), and then evaporated t dryness. Th residue was taken up in pyridine (5mL), bubbled with $H_2S$, and then evaporated to dryness. The residue was subjected to flash-column chromotography (silica gel, the first solvent system was ethyl acetate, the second solvent system was ethyl acetate/iso-propanol/$H_2O$ : 5/2/1). The ethyl acetate eluate contained compound (13) (260mg, 53%). The fractions with a positive ninhydrin reaction, collected from the second solvent system, were combined and evaporated to dryness to afford compound (12) (32mg, 6.5%).

MS (FAB), 431 ($M^+$ + 1), 414 ($M^+$ - NH2).
$^1$H-nmr ($CDCl_3$ + $CD_3OD$) δ (ppm) 1.96, 2.06, 2.08, 2.09 (s, 12H, Acetyl $CH_3$ x 4), 3.52 (dd, 1H, $J_{4,3}$ 5.5Hz, $J_{4,5}$ 4.5Hz, $H_4$), 3.80 (s, 3H, $COOCH_3$), 4.16 (dd, 1H, $J_{6,5}$ 10.2Hz, $J_{6,7}$ 2.3Hz, $H_6$), 4.17 (dd, 1H, $J_{9',9}$-12.4Hz, $J_{9',8}$ 7.3Hz, $H_{9'}$), 4.23 (dd, 1H, $J_{5,6}$ 10.2Hz, $J_{5,4}$ 4.5Hz, $H_5$), 4.73 (dd, 1H, $J_{9,9'}$-12.4Hz, $J_{9,8}$ 2.7Hz, $H_9$), 5.34 (ddd, 1H, $J_{8,7}$ 4.7Hz, $J_{8,9}$ 2.7Hz, $J_{8,9'}$ 7.3Hz, $H_8$), 5.45 (dd, 1H, $J_{7,6}$ 2.3Hz, $J_{7,8}$ 4.7Hz, $H_7$), 6.12 (d, 1H, $J_{3,4}$ 5.5Hz, $H_3$).

$^{13}$C-nmr ($CDCl_3$ + $CD_3OD$) δ (ppm) 20.7 ($CH_3C(O)O$-), 23.1($CH_3C(O)N$-), 43.8($C_5$), 46.2($C_4$), 52.4($COOCH_3$), 62.3($C_9$), 68.3, 71.8($C_7$, $C_8$), 73.0($C_6$), 111.5($C_3$), 143.8($C_2$), 162.4($C_1$), 170.3 & 170.8($CH_3CO$ x 4).

Compound (12) was stirred in anhydrous methanol (5mL) containing Amberlite IRA-400 (OH-) resin (100mg) at room temperature for 3 h. Following filtration, the filtrate was evaporated to dryness. The residue was dissolved in water (5mL) and adjusted to pH13 with 0.1M NaOH. The aqueous solution was stirred at room temperature for 2 hr and then neutralized with Dowex 50 x 8 ($H^+$) resin. After filtration, the filtrate was lyophilized to afford compound (14) (16mg, 70%), which was positive in the ninhydrin reaction.

mixture was stirred at room temperature f r 16 h and then was diluted with 0.2 M HCl (5 mL). Th mixture was stirred at room temperature for 48 h. To this reaction mixture were added ethyl acetate (50 mL) and 2 M HCl (1 mL). The organic layer was separated and washed with water (5 mL X 3), then evaporated to dryness. The residue was subjected to flash column-chromatography (silica gel, ethyl acetate/hexane=2/1). The fractions with $R_f$ value of 0.32 (ethylacetate/hexane=2/1 as developing solvent) were combined and evaporated to dryness to afford compound (15). (40 mg, 8.4%). The column was then eluted with ethyl acetate/methanol=10/1 to recover the starting material (2) (280 mg,56%). Compound (15) was isolated as a white foam substance.

MS (FAB) 457 ($M^+$+1), 414 ($M^+$-N3),
i.r. ($CHCl_3$) $cm^{-1}$ 2108 ($-N_3$), 1748 (carbonyl)
$^1$H-nmr ($CDCl_3$), δ (ppm)1.97, 2.04, 2.06, 2.07 (s,12H, acetyl $CH_3$ x 4), 3.82 (s, 3H, $COOCH_3$), 4.12~4.20 (m, 3H,$C_6$, $C_4$ & $C_9$), 4.51 (ddd, 1H, $J_{5,4}$ 4.4Hz, $J_{5,6}$ 10.7Hz, $J_{5,NH}$, 10.1Hz, $H_5$), 4.69 (dd, 1H, $J_{9,8}$ 2.6Hz, $J_{9,9'}$ 12.4Hz, $H_9$), 5.31 (m, 1H, $J_{8,7}$ 4.9Hz, $J_{8,9}$ 2.6Hz, $J_{8,9'}$ 7.0Hz, $H_8$), 5.45 (dd, 1H, $J_{7,6}$ 2.1Hz, $J_{7,8}$ 4.9Hz, $H_7$), 5.68 (d, 1H, $J_{NH,5}$ 10.1Hz,CONH), 6.15 (d, 1H, $J_{3,4}$ 5.7Hz, $H_3$)

$^{13}$C-nmr ($CDCl_3$) δ (ppm)
20.7, 20.8, ($CH_3CO$-O x 3), 23.1 (O $CH_3$ CO-NH), 44.8 ($C_5$), 52.6 ($COOCH_3$), 54.8 ($C_4$), 62.1 ($C_9$), 67.6, 71.3 ($C_7$, $C_8$), 73.5 ($C_6$), 104.5 ($C_3$), 146.3 ($C_2$), 161.5 ($C_1$), 169.9, 170.2, 170.5 (acetyl, -C=O X 4)

Compound (15) (40 mg, 0.088 mmol) was dissolved in anhydrous methanol (4 mL) containing sodium methoxide (6.4 mg, 0.12 mmol). The mixture was stirred at room temperature for 2 h and concentrated to dryness in vacuo to afford compound (16), which was then dissolved in water (3 mL), stirred at room temperature for 2 h, adjusted to pH 6~7 with Dowex 50 X 8 ($H^+$) resin, and then lyophilised to give the title compound (17) as a yellowish powder (25 mg, 83%).

MS (FAB) 445 ($M^+$+1), 414 ($M^+$ - $NHCH_3$)

$^1H$ - nmr ($CDCl_3$) δ (ppm)

1.95, 2.05, 2.06, 2.12 (s, 12H, acetyl $CH_3$ X 4), 2.45 (s, 3H, N-$CH_3$), 3.72 (dd, 1H, $J_{4,3}$ 2.3Hz, $J_{4,5}$ 9.2Hz, $H_4$), 3.89 (s, 3H, $COOCH_3$), 4.16 (dd, 1H, $J_{9',8}$ 7.2Hz, J9',9 12.3Hz, $H_{9'}$), 4.26 (ddd, 1H, $J_{5,4}$ 9.2Hz, $J_{5,NH}$ 9.1Hz, $J_{5,6}$ 9.0Hz, $H_5$), 4.36 (dd, 1H, $J_{6,5}$ 9.0Hz, $J_{6,7}$ 2.7Hz, $H_6$), 4.64 (dd, 1H, $J_{9,8}$ 2.9Hz, $J_{9,9'}$ 12.3Hz, $H_9$), 5.34 (m, 1H, $J_{8,7}$ 4.8Hz, $J_{8,9}$ 2.9Hz, $J_{8,9'}$ 7.2Hz, $H_8$), 5.51 (dd, 1H, $J_{7,6}$ 2.7Hz, $J_{7,8}$ 4.8Hz), 6.05 (d, 1H, $J_{3,4}$ 2.3Hz, $H_3$)

Compound (18) (25 mg, 0.056 mmol) was stirred in anhydrous methanol (5 mL) containing sodium methoxide (5.4 mg, 0.1 mmol) at room temperature for 2 h, then evaporated to dryness to give compound (19), which was redissolved in water (5 mL) and allowed to stand at room temperature for 2 h before being neutralized with Dowex 50 x 8 ($H^+$) resin. The filtrate was lyophilised to afford compound (20) (15 mg, 82%).

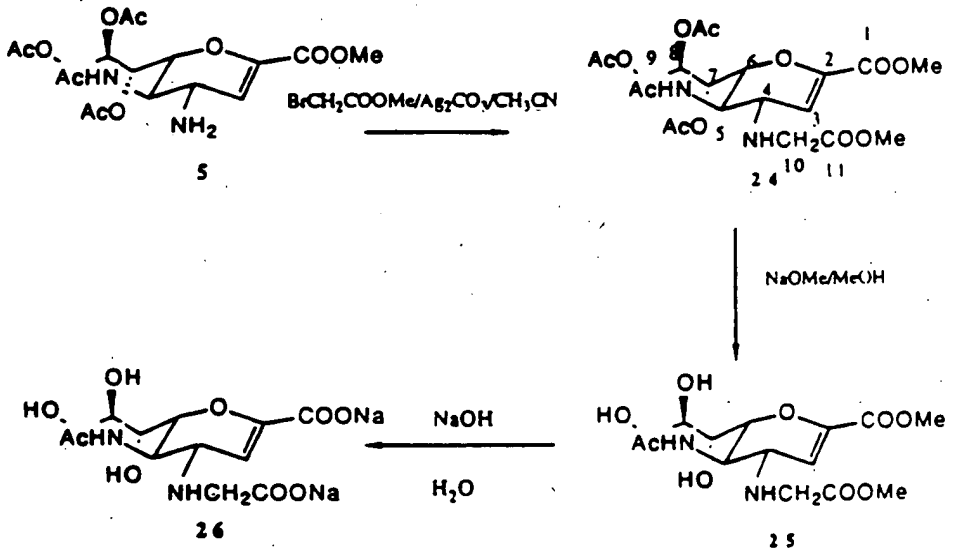
$^1H$-nmr ($D_2O$) δ (ppm)

1.94 (s, 3H, $CH_3CO$), 2.43 (s, 3H, N-$CH_3$), 3.5~4.3 (m, 7H, $H_4$, $H_5$, $H_6$, $H_7$, $H_8$, $H_9$ & $H_{9'}$), 5.65 (d, 1H, $J_{3,4}$ 2Hz, $H_3$)

Compound (21) (30 mg, 0.066 mmol) was stirred in anhydrous methanol (4 mL) containing dry Amberlite IRA 400 ($OH^-$) resin (90 mg) at room temperature for 3 h, then the resin filtered off. The filtrate and washings were combined and evaporated to dryness to afford compound (22) (20 mg), which was stirred in water (5 mL) at pH 12 at room temperature for 2 h, then was adjusted to pH 7.5 with Dowex 50 X 8 ($H^+$) before filtration. The filtrate was lyophilised to afford compound (23) (15 mg, 66%) as a white powder.

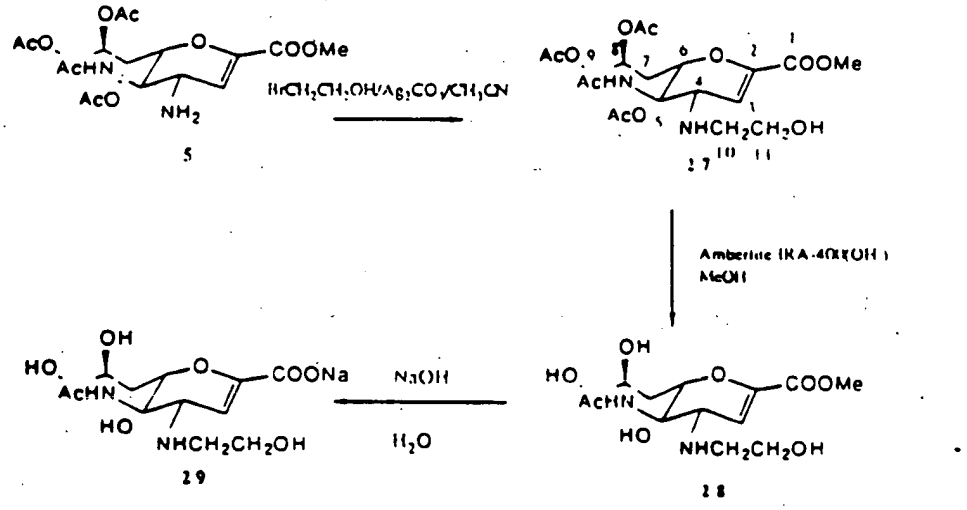
$^1$H-nmr ($D_2O$) δ (ppm)
1.97 (s, 3H, acetyl), 2.33 (s, 6H, $N(CH_3)_2$), 3.50-4.26 (m, 7H, $H_4$, $H_5$, $H_6$, $H_7$, $H_8$, $H_9$ & $H_{9'}$), 5.71 (d, $J_{3,4}$ 1.8Hz, $H_3$)

Example 10 Disodium 5-acetamido-4-N-oxycarbonylmethyl-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (26).




To a solution of methyl α-bromoacetate (36 mg, 0.23 mmol) and compound (5) (100 mg, 0.23 mmol) in acetonitrile (12 mL) was added silver carbonate (64 mg, 0.23 mmol). The mixture was stirred at room temperature for 16 h whilst shielded from light, then filtered. The filtrate was

Example 11 Sodium 5-acetamido-4-N-2'-hydroxyethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (29)



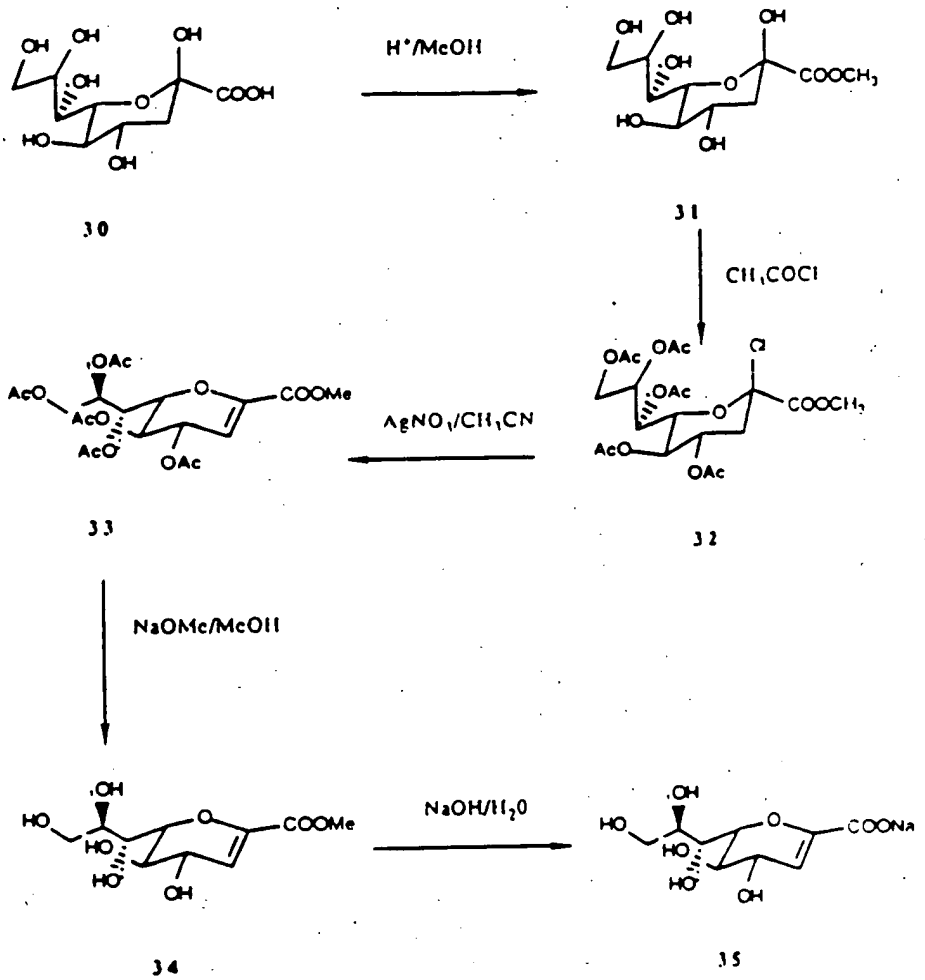

To a solution of bromoethanol (158 mg, 1.26 mmol) and compound (5) (84 mg, 0.195 mmol) in acetonitrile (10 mL) was added silver carbonate (100 mg, 0.36 mmol). The mixtur was protected from light and stirred at room temperature for 7 days. Then it was filtered off, the filtrate was evaporated to dryness. The residue was chromatographed on a silica gel column (ethyl acetate/isopropanol/water=5/2/1). Fractions with $R_f$ value of 0.4 were combined and evaporated to dryness to afford compound (27) (40 mL, 40%).

MS (FAB) 475 ($M^+$+1), 414 ($M^+$-$NHCH_2CH_2OH$)

$^1$H-nmr ($CDCl_3$) δ (ppm)

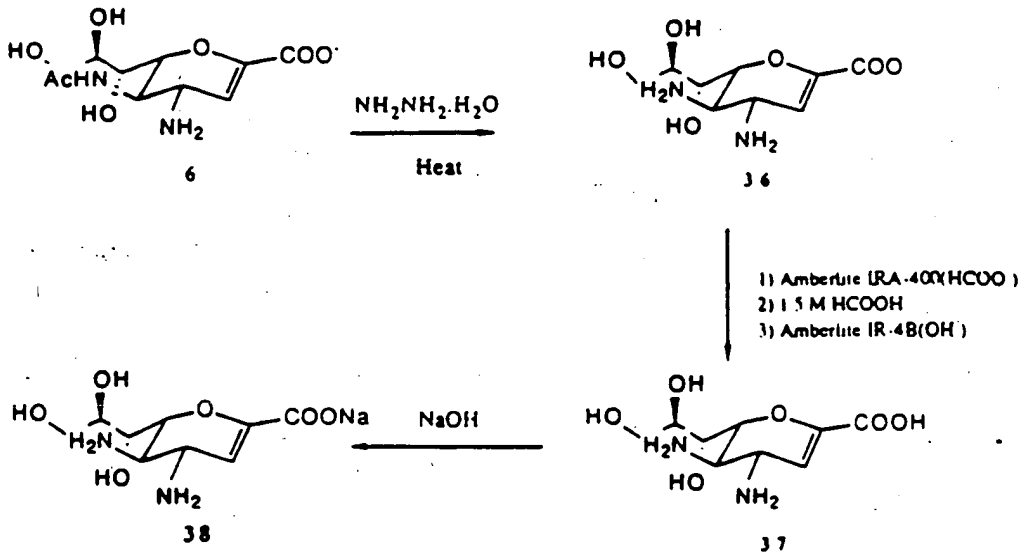
1.96, 2.05, 2.10 (s, 12H, acetyl $CH_3$ x 4), 2.29 (br. s, 2H, NH &OH), 2.76 (ABm, 2H, $H_{10}$ X 2), 3.47 (dd, 1H, $J_{4,3}$ 2.9Hz, $J_{4,5}$ 7.5Hz, $H_4$), 3.62 (t, 2H, $J_{11,10}$ 4.9Hz, $H_{11}$ x 2), 3.79 (s, 3H, $COOCH_3$), 4.15 (ddd, 1H, $J_{5,4}$ 7.5Hz, $J_{5,6}$ 8.4Hz, $J_{5,NH}$ 8.3Hz, $H_5$), 4.19 (dd, 1H, $J_{9',8}$ 7.5Hz, $J_{9',9}$ 12.3Hz $H_{9'}$), 4.29 (dd, 1H, $J_{6,5}$ 8.4Hz, $J_{6,7}$ 3.8Hz, $H_6$), 4.65 (dd, 1H, $J_{9,8}$ 2.9Hz, $J_{9,9'}$ 12.3Hz, $H_9$), 5.36 (m, 1H, $J_{8,7}$ 4Hz, $J_{8,9}$ 2.9Hz, $J_{8,9'}$ 7.5Hz, $H_8$), 5.55 (dd, 1H, $J_{7,6}$ 3.8Hz, $J_{7,8}$ 4Hz, $H_7$), 6.08 (d, 1H, $J_{3,4}$ 2.9Hz, $H_3$), 6.09 (d, 1H, $J_{NH,5}$ 8.3Hz, CONH)

Example 12 Sodium 2,-3-dideoxy-D-glycero-D-galacto-non-2-enopyranosonate (35)




Compound (30) (332 mg, 1.24 mmol) was stirred in anhydrous methanol (40 mL) containing Dowex 50 x 8 ($H^+$) resin (50 mg) at room temperature for 16 h before filtration. The filtrate was evaporated to dryness to give compound (31) (320 mg, 1.13 mmol, 91.5%), which was stirred in acetyl chloride (5 mL) at room temperature for 3 days then evaporated to dryness to afford Compound (32) (539 mg, 1.057 mmol, 93.6%). The residue was dissolved in acetonitrile (20 mL) containing silver nitrate (500 mg, 2.94 mmol) and potassium carbonate

Example 13 Sodium 4,5-Diamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (38)




A solution of compound (6) (125 mg, 0.40 mmol) in hydrazine hydrate (5 mL) under argon was heated at 85°C for 3 days, and the resulting mixture was vacuum evaporated to dryness. The residue was dissolved in water (15 mL) and passed through a column of Amberlite IRA-400 ($HCOO^-$), then eluted with 1.5 M HCOOH. The eluate (200 mL) was evaporated to dryness. The residue was chromatographed on silica gel deactivated with 10% water (developing solvent: isopropanol/water = 4/1). The fractions with $R_f$ value of 0.1 were combined and evaporated to dryness, then freeze-dried. The residue, compound (36), was dissolved in water (10 mL), passed through a small column of Amberlite IR-4B ($OH^-$)(10 mL). The effluent was evaporated to dryness to give compound (37), MS (FAB) of which was 249 ($M^+$+1). Compound (37) was dissolved in water and adjusted to pH 7.5 with 0.1 M NaOH, then freeze-dried to afford compound (38) (20 mg, 20%) as a white powder.

Exampl 15 Methyl 5-acetamido-9-azido-2,3,5,9-tetradeoxy-D-glycero-D-galacto-non-2- nopyranosonate (40)

Methyl 5-acetamido-2,3,5-trideoxy-9-(p-toluenesulphonyl)-D-glycero-D-galacto-non-2-enopyranosonate (39) (600 mg., 1.27 mmol) and lithium azide (186 mg., 3.80 mmol) were dissolved in dry DMF (20 mL) and the yellow homogenous solution heated to 80°C. After 2 h, further lithium azide (186 mg., 3.80 mmol) was added and the solution left at 80°C overnight. The solvent was removed by rotary evaporation and the remaining dark brown oil dissolved in pyridine (2 mL) and flash chromatographed ($SiO_2$, 5/2/1 EtOAc/i-PrOH/$H_2O$). The major product was compound (40) (370 mg., 88% yield) obtained as a white foam.

i.r.(KBr):$\nu_{max}$ ($cm^{-1}$) 3428 (s,OH), 2104 (s, $N_3$), 1730 (s, $CO_2CH_3$), 1656 (s, NHAc)
MS (FAB) : 331 ($M+H^+$)
$^1H$ nmr (300 MHz, $D_2O$): $\delta$ (ppm) = 1.94 (s, 3H, NHAc), 3.37 (dd, 1H, $H_9'$),
3.48 - 3.57 (m, 2H, $J_{8,9}$, 5.77, $H_8$ and $J_{9,9'}$ 13.16, $H_9$), 3.66 (s, 3H, $CO_2CH_3$), 3.91 - 3.98 (m, 2H, $H_5$ and $H_6$), 4.15 (d, 1H, $J_{7,8}$ 10.86, $H_7$), 4.38 (dd, 1H, $J_{4,5}$ 8.88, $H_4$), 5.91 (d, 1H, $J_{3,4}$ 2.44, $H_3$)

Example 16 Methyl 5,9-diacetamido-2,3,5,9-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (41).

Thiolacetic acid (130 mL, 1.82 mmol) was added to methyl 5-acetamido-9-azido-2,3,5,9-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (70 mg.,0.21 mmol) to give a pale yellow solution that was left to stir overnight at room temperature.

Excess thiolacetic acid was then evaporated off under low pressure and the remaining solid repeatedly treated with water followed by evaporation (3x3 mL). The remaining solid was dissolved in methanol (4 mL), filter d and the

A solution of methyl 5,9-diacetamido-2,3,5,9-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (41) (46 mg., 0.13 mmol) dissolved in 0.1M aq. sodium hydroxide (5 mL) was stirred at room temperature for 2.5 h. The solution was then adjusted to pH 5 with Dowex 50W-X8 ($H^+$), the resin filtered off and the filtrate lyophilized to give 40 mg. (91% yield) of compound (42) as a white powder.

i.r. (KBr) : $\nu_{max}$ ($cm^{-1}$) 3376 (s, OH), 1652 (s, NHAc)
MS (FAB) : 333 ($M+H^+$)
$^1H$ nmr (300 MHz, $D_2O$) : $\delta$ (ppm) = 1.89 (s, 3H, NHAc), 1.93 (s, 3H, NHAc),
3.15 (dd, 1H, $H_{9'}$), 3.40 (d, 1H, $H_6$), 3.48 (dd, 1H, $J_{9,9'}$ 14.18, $H_9$),
3.82 (m, 1H, $J_{8,9}$ 3.01, $J_{8,9'}$ 7.43, $H_8$), 3.94 (dd, 1H, $J_{5,6}$ 10.42, $H_5$), 4.13 (d, 1H, $J_{7,8}$ 10.91, $H_7$), 4.36 (dd, 1H, $J_{4,5}$ 8.80, $H_4$), 5.81 (d, 1H, $J_{3,4}$ 2.41, $H_3$)

Example 18 Methyl 5-acetamido-9-cyano-2,3,5,9-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (43)

A solution of methyl 5-acetamido-2,3,5-trideoxy-9-(p-toluenesulphonyl)-D-glycero-D-galacto-non-2-enopyranosonate (39) (80 mg., 0.17 mmol), tert-butylammonium cyanide (2 mg) and sodium cyanide (12 mg., 0.25 mmol) in dry DMSO (1.25 mL) was stirred at room temperature for 5 days.

Workup by preparative thin layer chromatography ($SiO_2$, 20 cm. x 20 cm. x 2 mm. eluted with EtOAc/ i-PrOH/$H_2O$, 5/2/1) gave as the major component 30 mg. (61% yield) of compound (43) as a cream coloured powder.

($R_f$=0.74).
i.r.(KBR) : $\nu_{max}$ ($cm^{-1}$) 3440 (s, OH), 2256 (w, CN), 1726 (s, $CO_2CH_3$),
1638 (s, NHAc)
MS (FAB) : 315 ($M+H^+$)
$^1H$ nmr (300mHz, $D_2O$) : $\delta$ (ppm) = 1.92 (s, 3H, NHAc), 2.75

i.r. (KBr) : $\nu_{max}$ ($cm^{-1}$) 3370 (s,OH), 2254 (w, CN), 1656 (s, NHAc)

MS (FAB) : 301 ($M+H^+$)

$^1H$ nmr (300MHz, $D_2O$) : $\delta$ (ppm) = 1.98 (s, 3H, NHAc), 2.70 (dd, 1H, $H_9'$),

2.88 (dd, 1H, $J_{9,9'}$ 17.27, $H_9$), 3.48 (d, 1H, $H_6$), 3.97 (dd, 1H, $J_{5,6}$ 9.84, $H_5$), 4.09-4.24 (m, 2H, $H_7$ and $H_8$,$J_{8,9}$ 3.90,$J_{8,9'}$ 6.53), 4.41 (dd, 1H, $J_{4,5}$ 8.87, $H_4$), 5.80 (d, 1H, $J_{3,4}$ 2.42, $H_3$)

Example 20 Inhibition of Influenza Virus Neuraminidase

An in vitro bioassay of the above-described compounds against N2 influenza virus neuraminidase was conducted, following Warner and O'Brien, Biochemistry, 1979 18 2783-2787. For comparison, with the same assay the $K_i$ for 2-deoxy-N-acety-α-D-neuraminic acid was determined to be 3 x $10^{-4}$ M.

Values for $K_i$ were measured via a spectrofluorometric technique which uses the fluorogenic substrate 4-methylumbelliferyl N-acetylneuraminic acid (MUN), as described by Meyers et al., Anal. Biochem. 1980 101 166-174. For both enzymes, the assay mixture contained test compound at several concentrations between 0 and 2 mM, and approximately 1 mU enzyme in buffer (32.5 mM MES, 4 mM $CaCl_2$, pH 6.5 for N2; 32.5 mM acetate, 4 mM $CaCl_2$, pH 5.5 for V. cholerae neuraminidase).

The reaction was started by the addition of MUN to final concentrations of 75 or 40 μM. After 5 minutes at 37°C, 2.4 ml 0.1 M glycine-NaOH, pH 10.2 was added to 0.1 ml reaction mixture to terminate the reaction. Fluorescence was read at excitation 365 nm, emission 450 nm, and appropriate MUN blanks (containing no enzyme) were subtracted from readings. The $K_i$ was estimated by Dixon plots (1/fluorescence versus compound concentration). Results are summarized in Table 1, and unless otherwise stated, refer to inhibition of N2 neuraminidas .

| | |
|---|---|
| | $4.5 \times 10^{-4}$ (*V. cholerae* neuraminidase; pH 5.8) |
| | $> 10^{-2}$ (sheep neuraminidase; pH 4.5) |
| sodium 5-acetamido-4-N,N-diallylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (9) | $4 \times 10^{-6}$ |
| sodium 5-acetamido-4-N-allylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranoonate (11) | $2.5 \times 10^{-6}$ (N2 and N9 neuraminidase) |
| Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-talo-non-2-enopyranosonate (12) | approx. $3 \times 10^{-3}$ |
| Methyl 7,8,9-tri-O-acetyl-2,3,5-trideoxy-4',5'-dihydro-2'-methyl-oxazolo [5,4-d] D-glycero-D-talo-non-2-enopyranosonate (13) | $3 \times 10^{-5}$ |
| sodium 5-acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-talo-non-2-enopyranosonate (14) | $1 \times 10^{-7}$ (N2 and N9 neuraminidase) |
| Sodium 5-acetamido-4-azido-2,3,4,5-tetradeoxy-D-glycero-D-talo-non-2-enopyranosonate (17) | $2.8 \times 10^{-5}$ |

| | | |
|---|---|---|
| Methyl 5-acetamido-9-cyano-2,3,5,9-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (43) | approx. 3 | x $10^{-3}$ |
| 5-Acetamido-9-cyano-2,3,5,9-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid (44) | 3 | x $10^{-6}$ |

TABLE 2

| Compound | $IC_{50}$ Plaque Reduction ($\mu$M) Influenza A | Influenza B |
|---|---|---|
| Sodium 5-Acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-amino-Neu5Ac2en) (6) | 1.6 | 1.6 |
| Sodium 5-Acetamido-4-amino-2,3,4,5-tetra deoxy-D-glycero-D-talo-non-2-enopyranosonate (14). | 3.0 | 1.2 |
| Ammonium 5-Acetamido-4-guanidino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (7) | 1.6 | 1.6 |
| Sodium 5-acetamido-4-N-2'-hydroxyethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (29) | 60 | 7 |
| Sodium 5-acetamido-4-N-allyl-N-hydroxy-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (45) | 4.7 | 2.7 |
| Sodium 4,5-Diamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (38) | 11 | 6.8 |

Table 3

Efficacy in Influenza Virus Infected Mice

| Experiment Number | Compound | Dose (mg/kg body weight) | % AUC |
|---|---|---|---|
| 1 | Sodium 5-Acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-amino-Neu5Ac2en) (6) | 25 | 0.06 |
| | Amantadine | 25 | 0.08 |
| | DANA | 25 | 0.18 |
| 2 | Ammonium 5-Acetamido-4-guanidino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (7) | 12.5 | 0.03 |
| | Ribavirin | 25 | 29.8 |
| | Amantadine | 25 | 0.2 |
| | | 12.5 | 0.03 |
| | DANA | 12.5 | 2.0 |
| 3 | Sodium 5-Acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-talo-non-2-enopyranosonate (14) | 12.5 | 21.1 |
| | Amantadine | 12.5 | 8.8 |
| | DANA | 12.5 | 48.0 |

It will b clearly understood that the invention in its general aspect is not limited to the specific details referred to hereinabove.

group are the same or different,

and pharmaceutically acceptable salts or derivatives thereof,

provided that in general formula (I)

(i) when $R^3$ or $R^{3'}$ is $OR^6$ or hydrogen, and A is oxygen or sulphur, then said compound cannot have both

(a) an $R^2$ that is hydrogen and

(b) an $R^4$ that is NH-acyl, and

(ii) $R^6$ represents a covalent bond when Y is hydrogen, and that in general formula (Ia),

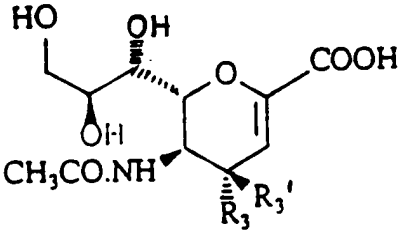
(i) when $R^3$ or $R^{3'}$ is $OR^6$ or hydrogen, and A is nitrogen, then said compound cannot have both

(a) an $R^2$ that is hydrogen, and

(b) an $R^4$ that is NH-acyl, and

(ii) $R^6$ represents a covalent bond when Y is hydrogen.

2. A compound as claimed in Claim 1 wherein the compound is a compound of formula (II)




(II)

wherein $R^3$ is hydrogen or $R^{3'}$ and $R^{3'}$ is $-N_3$, $-CN$, $-CH_2NH_2$, or $-N.R^8.R^9$;

$R^8$ and $R^9$ are the same or different, and each denotes hydrogen, a linear or cyclic alkyl group of 1 to 6 carbon atoms, an acyl or substituted acyl group of 1 to 6 carbon atoms, $-C.(NH).NH_2$, $-CH_2.COOH$, $CH_2-CH_2-OH$ or $-CH_2.CH.(R^{10})(R^{11})$,

$R^{10}$ and $R^{11}$ may be the same or different, and each denotes oxygen or $R^{12}N=$, and

$R^{12}$ denotes hydrogen, $-OH$, $-OCH_3$, $-NH_2$, or $(CH_3)_2N-$or a pharmaceutically acceptable salt or derivative thereof.

3. A compound as claimed in Claim 1 or Claim 2 which is a compound of formula (Ib)

7. A compound as claimed in Claim 1 and selected from th group consisting of:-

Sodium 5-acetamido-4-azido-2,3,4,5-tetradeoxy-D-galacto-non-2-enopyranosonate (4-Azido-Neu5Ac2en);

Sodium 5-acetamido-4-N,N-diallylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate;

Sodium 5-acetamido-4-N-allylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-epi-amino-Neu4Ac2en);

Sodium 5-acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-talo-non-2-enopyranosonate;

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-N,N-diallylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N,N-diallylamino-Neu5,7,8,9$Ac_4$2en1Me);

Sodium 5-acetamido-4-N,N-diallylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate;

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-N-allylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N-allylamino-Neu5,7,8,9$Ac_4$2en1Me);

Sodium 5-acetamido-4-N,N-dimethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate;

Sodium 2,-3-dideoxy-D-glycero-D-galacto-non-2-enopyranosonate;

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-azido-2,3,4,5-tetradeoxy-D-glycero-D-talo-non-2-enopyranosonate (4-epi-azidoNeu5,7,8,9$Ac_4$2en1Me);

8. 5-acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid and pharmaceutically acceptable salts and derivatives thereof.

9. Sodium 5-acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate.

10. 5-acetamido-4-guanidino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid and pharmaceutically acceptable salts and derivatives thereof.

11. Ammonium 5-acetamido-4-guanidino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate.

12. A pharmaceutical formulation comprising a compound

$R^4$ denotes $NHR^6$, $SR^6$, $OR^6$, $COOR^6$, $NO_2$, $C(R^6)_3$, $CH_2COOR^6$, $CH_2NO_2$ or $CH_2NHR^6$, and

$R^5$ denotes $CH_2YR^6$, $CHYR^6CH_2YR^6$ or $CHYR^6CHYR^6CH_2YR^6$, where Y is O, S, NH or H, and successive Y moieties in an $R^5$ group are the same or different,

or a pharmaceutically acceptable salt or derivative thereof, together with a pharmaceutically acceptable carrier therefor.

13. A pharmaceutical formulation as claimed in Claim 12 wherein the compound of formula (I) is a compound as claimed in any one of Claims 1 to 9.

14. A pharmaceutical formulation as claimed in Claim 12 or Claim 13 wherein the formulation is adapted for intranasal administration.

15. Use of a compound of formula (I) as defined in Claim 12 in the manufacture of a medicament for the treatment of a viral infection.

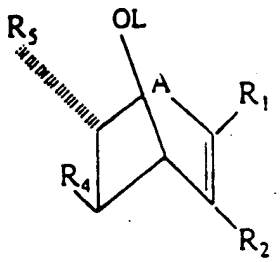
16. Use of a compound as claimed in Claim 15 wherein the viral infection is influenza.

17. A method for the treatment of a mammal including man suffering from a viral infection which comprises the step of administering to said mammal an effective amount of a compound of formula (I) as defined in Claim 11.

18. A method as claimed in Claim 17 wherein the viral infection is influenza.

19. A method for the preparation of a compound of formula (I) as defined in Claim 1 which comprises the steps of

(A) reaction of a compound of formula (III)

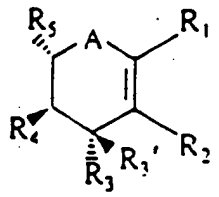



(III)

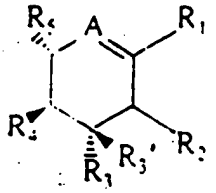
AMENDED CLAIMS

[received by the International Bureau
on 1 October 1991 (01.10.91):
original claim 13 cancelled:
original claims 1,3,7 and 12 amended:
new claims 10, 13-15, 19 and 24-37 added:
other claims unchanged (9 pages)]

1. A compound of formula (I) or formula (Ia)



(I)



(Ia)

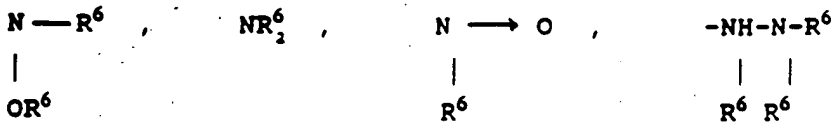
where in general formula (I), A is oxygen, carbon or sulphur; and in general formula (Ia), A is nitrogen or carbon;

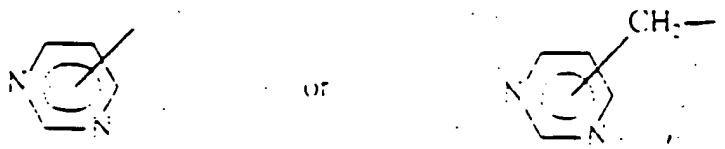
$R^1$ denotes COOH, $P(O)(OH)_2$, $NO_2$, SOOH, $SO_3H$, tetrazol, $CH_2CHO$, CHO or $CH(CHO)_2$,

$R^2$ denotes H, $OR^6$, F, Cl, Br, CN, $NHR^6$, $SR^6$ or $CH_2X$, wherein X is $NHR^6$, halogen or $OR^6$ and

$R^6$ is hydrogen; an acyl group having 1 to 4 carbon atoms; a linear or cyclic alkyl group having 1 to 6 carbon atoms, or a halogen-substituted analogue thereof; an allyl group or an unsubstituted aryl group or an aryl substituted by a halogen, an OH group, an $NO_2$ group, an $NH_2$ group or a COOH group,

$R^3$ and $R^{3'}$ are the same or different, and each denotes hydrogen, CN, $NHR^6$, $N_3$, $SR^6$, $=N-OR^6$, $OR^6$, guanidino,





$R^4$ denotes $NHR^6$, $SR^6$, $OR^6$, $COOR^6$, $NO_2$, $C(R^6)_3$, $CH_2COOR^6$, $CH_2NO_2$ or $CH_2NHR^6$, and

$R^5$ denotes $CH_2YR^6$, $CHYR^6CH_2YR^6$ or $CHYR^6CHYR^6CH_2YR^6$, where Y is O, S, NH or H, and successive Y moieties in an $R^5$

or a pharmaceutically acceptable salt or derivative thereof.

3. A compound as claimed in Claim 1 or Claim 2 wherein $R^3$ is $NHR^6$.

4. A compound as claimed in any one of Claims 1 to 3 wherein $R^3$ is

$NH_2$ or $NH\text{-}C(=NH)\text{-}NH_2$.

5. A compound as claimed in Claim 1 and selected from the group consisting of:-

Sodium 5-acetamido-4-azido-2,3,4,5-tetradeoxy-D-galacto-non-2-enopyranosonate (4-Azido-Neu5Ac2en);

Sodium 5-acetamido-4-N,N-diallylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate;

Sodium 5-acetamido-4-N-allylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate;

Sodium 5-acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-talo-non-2-enopyranosonate; (4-epi-amino-Neu4Ac2en);

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-N,N-diallylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N,N-diallylamino-Neu5,7,8,9$Ac_3$2en1Me);

Sodium 5-acetamido-4-N,N-diallylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate;

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-N-allylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N-allylamino-Neu5,7,8,9$Ac_4$2en1Me);

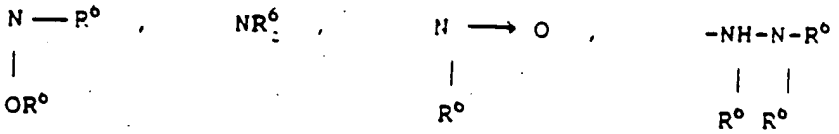
Sodium 5-acetamido-4-N,N-dimethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate;

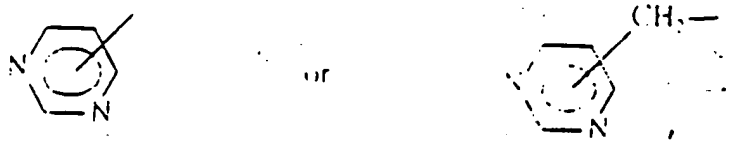
Sodium 2,-3-dideoxy-D-glycero-D-galacto-non-2-enopyranosonate; and

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-azido-2,3,4,5-tetradeoxy-D-glycero-D-talo-non-2-enopyranosonate (4-epi-azidoNeu5,7,8,9$Ac_3$2en1Me).

6. A pharmaceutical formulation comprising a compound of formula (I) or (Ia) as defined in any one of Claims 1 to 5, or a pharmaceutically acceptable salt or derivative

$R^3$ and $R^{3'}$ are the same or different, and each denotes hydrogen, CN, $NHR^6$, $N_3$, $SR^6$, $=N-OR^6$, $OR^6$, gaunidino,





$R^4$ denotes $NHR^6$, $SR^6$, $OR^6$, $COOR^6$, $NO_2$, $C(R^6)_3$, $CH_2COOR^6$, $CH_2NO_2$ or $CH_2NHR^6$, and

$R^5$ denotes $CH_2YR^6$, $CHR^6CH_2YR^6$ or $CHYR^6CHYR^6CH_2YR^6$, where Y is O, S, NH or H, and successive Y moieties in an $R^5$ group are the same or different,

or a pharmaceutically acceptable salt or derivative thereof.

11. A method for the treatment of a mammal including man suffering from a viral infection which comprises the step of administering to said mammal an effective amount of a compound of formula (I) or (Ia) as defined in Claim 1.

12. A method as claimed in Claim 11 wherein the viral infection is influenza.

13. A method as claimed in either Claim 10 or Claim 11 wherein the infection is by a respiratory virus.

14. A method as claimed in any one of Claims 10 to 13 wherein the active ingredient is administered to the respiratory tract.

15. A method as claimed in any one of Claims 10 to 14 wherein the active ingredient is administered intranasally.

16. A method for the preparation of a compound of formula (I) as defined in Claim 1 which comprises the steps of

(A) reaction of a compound, of formula (III)

$R^{4b}$ is $NHCOR^{9b}$ where $R^{9b}$ is hydrogen, substituted or unsubstituted $C_{1-4}$alkyl or aryl,

or a pharmaceutically acceptable salt or derivative thereof.

18. A compound as claimed in Claim 3 wherein $R^{3b}$ is $NR^{6b}R^{7b}$.

19. A compound as claimed in Claim 17 or Claim 18 wherein $R^{3b}$ is $NH_2$ or $NHC(=NH)NH_2$.

20. 5-acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid and pharmaceutically acceptable salts and derivatives thereof.

21. Sodium 5-acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate.

22. 5-acetamido-4-guanidino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid and pharmaceutically acceptable salts and derivatives thereof.

23. Ammonium 5-acetamido-4-guanidino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate.

24. A pharmaceutical formulation comprising a compound as claimed in any one of Claims 17 to 23 as active ingredient together with a pharmaceutically acceptable carrier therefor.

25. A pharmaceutical formulation suitable for intranasal administration comprising a compound as claimed in any one of Claims 17 to 23 as active ingredient together with a pharmaceutically acceptable carrier therefor.

26. A pharmaceutical formulation as claimed in Claim 24 or Claim 25 wherein the active ingredient is 5-acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid or a pharmaceutically acceptable salt thereof.

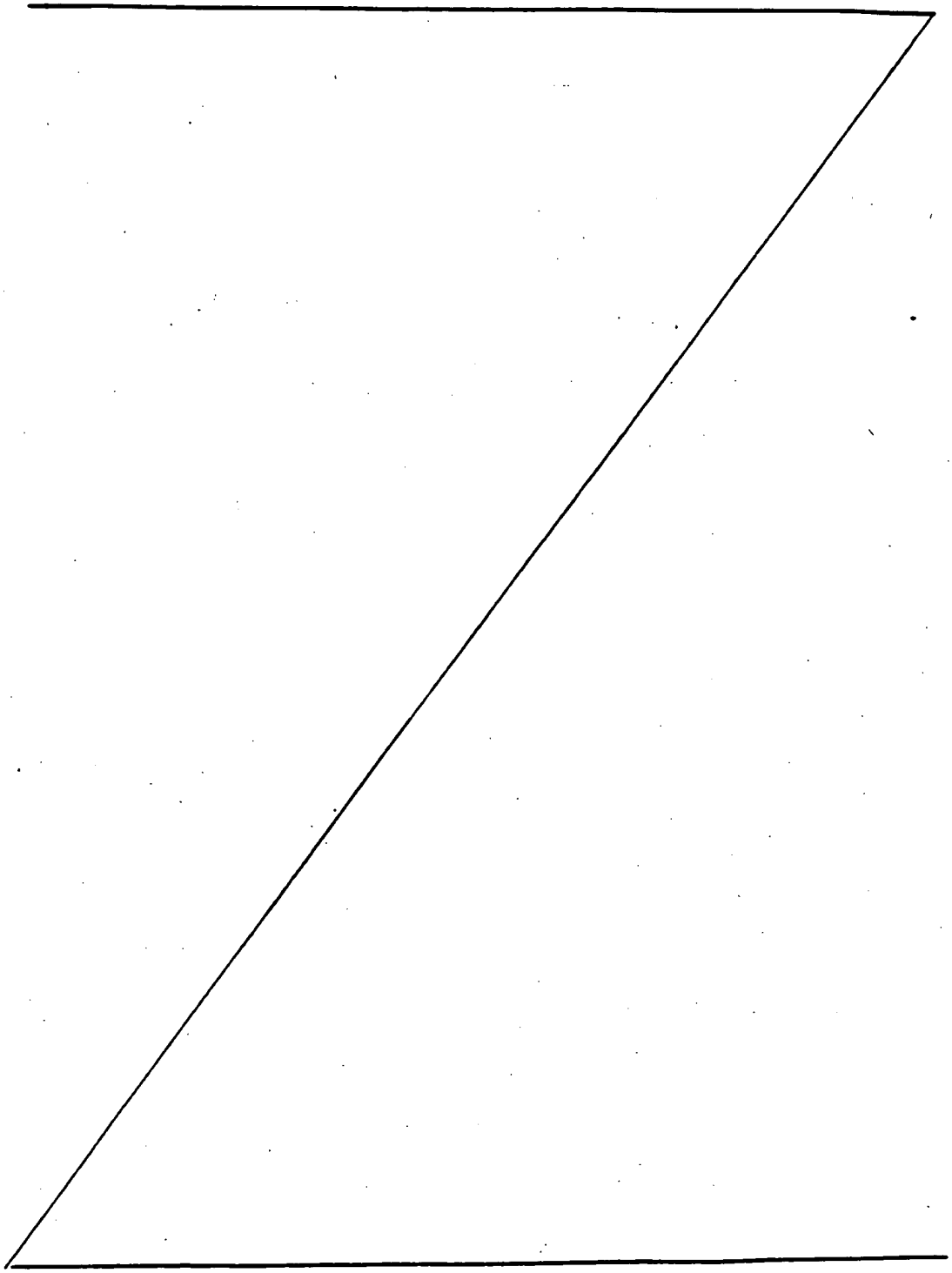
27. A pharmaceutical formulation as claimed in Claim 24 or Claim 25 wherein the active ingredient is 5-acetamido-4-guanidino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid or a pharmaceutically acceptable salt thereof.

28. A method for the treatment of a mammal including man suffering from a viral infection comprising administration of an effective amount of a compound as

and if necessary subjecting the resulting compound to one or two further reactions c mprising:

(i) removing any protecting groups;

(ii) converting a compound of formula (Ib) or a salt thereof into a pharmaceutically acceptable salt thereof.



# INTERNATIONAL SEARCH REPORT

International Application No. PCT/AU 91/00161

## I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 6

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.[5] C07D 309/30; 309/28; 309/26; 309/22; C07D 309/20; 335/02; 211/78; 211/74; 211/72; 211/70

## II. FIELDS SEARCHED

Minimum Documentation Searched 7

| Classification System | Classification Symbols |
|---|---|
| IPC | C07D 309/30; 309/28; 309/26; 309/22; 309/20; 335/02; 211/78; 211/74; 211/72; 211/70. Chemical Abstracts - ON-LINE* database for substructure search of pyran, thiopyran, piperidine and cyclohexane, with a glycerol moiety at position-2 optionally having a double bond at positions 5 or 6. |

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 8

AU : IPC as above

## III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

| Category* | Citation of Document, 11 with indication, where appropriate, of the relevant passages 12 | Relevant to Claim No 13 |
|---|---|---|
| A | DE,A, 1493249 (Dr Karl Thomae GmbH) Claim 1 | 1-6 |
| A | Tetrahedron Letters, vol. 28, No. 2, 1987, pages 191-194, published by Pergamon Journals Ltd. (U.K.) H. Mack and R. Brossmer, "Synthesis of 6-thiosialic acids and 6-thio-N-acetyl-D-neuraminic acid" | 1-6 |
| X | Chemical and Pharmaceutical Bulletin, vol. 36, no. 12, 1988, pages 4807-4813 published by Pharmaceutical Society of Japan M. Nakamura et al "Studies on sialic acids, XV, Synthesis of $\alpha$, and $\beta$ -O-glycosides of 3-deoxy-D-glycero-D-galacto-2-nonulopyranosonic acids (KDN)", page 4803, compound (13) | 1 |

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

## IV. CERTIFICATION

| Date of the Actual Completion of the International Search | Date of Mailing of this International Search Report |
|---|---|
| | 2 August 91 |
| **International Searching Authority** | **Signature of Authorized Officer** |
| Australian Patent Office | C.A. ERICK |

Form PCT/ISA/210 (second sheet) (January 1985)

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU 91/00161

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent Document Cited in Search Report | Patent Family Members |
|---|---|
| DE 1439249 | |

END OF ANNEX